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Title page:

Causes of infective endocarditis in the Western Cape, South Africa: A prospective cohort study utilising a set protocol for organism detection and central decision making by an Endocarditis team

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Abstract

Background:

Blood culture negative infective endocarditis(BCNIE) poses both a diagnostic and therapeutic challenge. High rates of BCNIE reported in South Africa have been attributed to antibiotic use prior to blood culture sampling.

Objectives:

To identify the causes of infective endocarditis, in particular causes of BCNIE.

Design:

Prospective cohort study.

Methods:

The Tygerberg Endocarditis Cohort(TEC) study prospectively enrolled patients with infective endocarditis(IE) between November 2019 and February 2021. A set protocol for organism detection with management of patients by an Endocarditis Team was employed. This prospective cohort was compared to a retrospective cohort of patients with IE admitted between January 2017 and December 2018.

Results:

Hundred and forty patients with IE were included, with 75 and 65 patients in the retrospective and prospective cohorts respectively. Baseline demographic characteristics were similar with a mean age of 39,6 years and male predominance(male sex = 67.1) The rate of BCNIE was lower in the prospective group(28/65 or 43.1%) compared to the retrospective group(47/75 or 62.7%;p=0.039). The BCNIE in-hospital mortality rate in the retrospective cohort was 23.4% compared to 14.2% in the prospective cohort(p=0.35). A cause was identified (including non-culture techniques) in 86.2% of patients in the prospective cohort, with *Staphylococcus aureus*(26.2%), *Bartonella* species(20%) and the viridans group of streptococci(15.3%) being most common.

Conclusion:

The introduction of a set protocol for organism detection, managed by an Endocarditis Team, has identified *Staphylococcus aureus* as the most common cause of IE and identified non-culturable organisms, in particular *Bartonella quintana*, as an important cause of BCNIE. A reduction in in-hospital mortality in patients with BCNIE was observed, but did not reach statistical significance.

Strengths and limitations of this study

- This is the first prospective cohort study that has evaluated the impact of an Endocarditis Team, with a set protocol for organism detection, on patients with IE in South Africa
- A comprehensive protocol for organism detection was employed to minimize the rate of BCNIE and identify non-culturable organisms
- Causative organisms of IE, in particular BCNIE, varies geographically. This may limit the generalisability of this data

Introduction

Infective endocarditis (IE) is an infection involving the endocardial surface of the heart. This can affect native heart valves (native valve endocarditis or NVE), prosthetic valves (prosthetic valve endocarditis or PVE), non-valvular endocardial surfaces (such as IE affecting ventricular septal defects) or any non-valvular prosthetic devices.(1–4) Identification of the causative organism via blood cultures is fundamental to the diagnosis and treatment of IE.(2,4) Blood cultures that fail to identify the causative organism in patients with clinical and / or imaging evidence of IE - so called blood culture negative IE (BCNIE) - pose both a diagnostic and therapeutic challenge to the treating physician. BCNIE has been associated with worse outcomes compared to patients with blood culture

positive IE (BCPIE), although more recent reports have demonstrated equivalent outcomes.(5–7) It is important to note that the proportion of patients with BCNIE has decreased, which is likely due to a decrease in antibiotic use prior to blood culture collection.(6,7) Although BCNIE is still diagnosed in a significant proportion of patients with IE, in the majority of patients the organism or cause is identified via non-culture-dependant methods. The identification of organisms responsible for BCNIE (and thus appropriate treatment) has coincided with more equal outcomes when comparing BCNIE to BCPIE presumably due to more targeted therapy.(7,8) BCNIE was previously mainly attributed to sterilized blood cultures due to antibiotic use prior to acquisition of adequate blood culture samples. Although this is still a contributor, IE caused by organisms that are either intracellular or difficult to culture with standard culture methods, have emerged as an important cause of BCNIE.(7,9,10) These organisms vary according to geographic region with *Coxiella burnettii* more common in European cohorts in contrast to African cohorts demonstrating *Bartonella* species as the most common cause of BCNIE.(9,11) Non-infectious causes e.g. non-bacterial thrombotic endocarditis are rare causes of BCNIE.(8,12,13)

Very high rates of BCNIE have been reported in South Africa varying from 40-65%.(8,12,13) This was attributed to high rates of antibiotic use prior to blood culture sampling (25%-52%), although no systematic approach to organism detection was employed and thus no information is available about the other causes of BCNIE in South Africa.(2,8,13) Our group recently reported the emergence and typical clinical and imaging findings of *Bartonella* species as a cause of BCNIE in South Africa.(14)

We postulated that the implementation of a set protocol for organism detection and management of patients with IE by an Endocarditis Team would identify causes of BCNIE and improve the short-term outcome of patients with both BCPIE and BCNIE.

Methods

All patients presenting to the Division of Cardiology, Department of Medicine at Tygerberg Hospital in Cape Town, South Africa, with IE (2) between November 2019 and February 2021 were prospectively included in the Tygerberg Endocarditis Cohort (TEC) study. Patients with known or newly diagnosed malignancy were excluded from this study.

The Division of Cardiology at Tygerberg Hospital is a public sector tertiary referral centre that serves a population of approximately 2.4 million people.(15) All patients presenting with features of IE to hospitals within the referral network are referred to Tygerberg Hospital for definitive care.

All patients were managed by an Endocarditis Team (2) which fulfilled all the criteria as set out by current guidelines. All patients underwent standard transthoracic echocardiography (TTE) and transoesophageal echocardiography (TEE) in the absence of identifiable contra-indications to TEE. Additional imaging was performed at the discretion of the Endocarditis Team.

A stepwise protocol for organism detection (supplementary file A) was utilised to identify the causative organisms of IE and to minimize the incidence of BCNIE (Figure 1). A minimum of three sets of blood cultures (BacT/ALERT, bioMérieux, Marcy l'Etoile, France), including one aerobic and one anaerobic bottle per set, were required, with repeated cultures if clinical

features of infection persisted. Further management and analysis of the samples were done according to current published guidelines.(2,14) Patients without an identified organism after five days, using standard culture techniques, were defined as BCNIE.

All BCNIE patients underwent venous blood analysis (all test performed in parallel) for further testing, including:

- Testing for antinuclear antibodies (ANA) and anti-cardiolipin antibodies (ACLA)
- Serology was performed using indirect immunofluorescence assays (IFA) for detection of IgM and IgG antibodies to *Bartonella henselae* and *Bartonella quintana* (FOCUS Diagnostics, Cypress, CA, USA). Specific antibodies to *Coxiella burnetii* were also determined by IFA. Enzyme immunoassays (EIA) were performed to detect IgM and IgG antibodies to *Brucella* species, *Legionella pneumophila* (EUROIMMUN, Lübeck, Germany) and *Mycoplasma pneumoniae* (EUROIMMUN, Lübeck, Germany).
- Direct polymerase chain reaction (PCR) was performed on blood culture bottles for detection of the universal bacterial 16S rRNA and ITS2 for fungi, followed by sequencing to identify the amplified DNA product
- BACTEC Myco/F Lytic vials (Becton Dickinson, San Jose, CA, USA) were collected for the isolation of Mycobacteria, including *Mycobacterium tuberculosis* (MTB) and non-tuberculous Mycobacteria

A sample of heart valve tissue was collected from all patients who required surgery and this was submitted for:

- Bacterial and fungal culture
- Broad range PCR with 16S rRNA for bacteria and ITS2 for fungi, followed by sequencing to identify the amplified DNA product

- Histopathologic examination to detect bacteria and fungi, as well as histopathological features of IE

All patients were managed according to current guidelines by the Endocarditis Team and prospectively followed.(2,14) Baseline demographic and clinical features, results of special investigations including microbiological evaluation and imaging findings were documented on all patients. Treatment strategy, including specific antimicrobial therapy and surgical interventions were documented. Patients were followed till hospital discharge and all major adverse events (death, embolic events, renal failure) were recorded.

To evaluate the impact of this strategy, the prospective cohort was compared with a retrospective cohort that comprised of patients with IE admitted to Tygerberg Hospital from January 2017 to December 2018. In this latter cohort, diagnostic evaluation and treatment was not standardized and at the discretion of the managing physician (rather than formalised in an Endocarditis Team) and without a step wise protocol for organism detection. Serology, blood rRNA PCR on blood and heart valve PCR was rarely performed. All retrospective data was collected from patient folders, echocardiography-, laboratory- and surgical databases.

Patients who presented within the Tygerberg Hospital referral network but surmised due to IE before referral to Tygerberg Hospital was included to minimise selection bias.

Statistical analysis

Statistical analysis was done using SPSS v27 for iOS and JASP (Version 0.14.1) for iOS. Descriptive statistics were calculated, nominal data was compared via cross tabulation and Chi-square tests, parametric data was compared using independent-sample T-tests (Cohen's d) and non-parametric data was compared using independent-samples T-test (Mann-Whitney U or Kruskal-Wallis 1-way ANOVA).

Ethical considerations

This study was approved by the Health Research Ethics Committee (HREC) of Stellenbosch University (Project numbers S19/08/162 and S19/10/234) and performed in accordance with the Declaration of Helsinki (2013 version). All patients in the prospective cohort signed written informed consent; a waiver of consent was obtained from HREC to include patients in the retrospective cohort.

Patient and public involvement

It was not possible to involve patients or the public in the design, or conduct, or reporting, or dissemination plans of our research.

Results

A total of 140 patients with IE were included, with 75 and 65 patients in the retrospective and prospective cohorts respectively. The baseline characteristics of patients in both cohorts are summarized in Table 1. The mean age was 39,6 years with a male predominance (male sex = 67.1%). Fourteen of the 75 patients (21.5%) in the retrospective cohort were HIV-positive compared to 18 of 65 (29%) in the prospective cohort (p=0.21). There was no difference in absolute CD4 count (442cells/µl vs 402cells/µl; p=0.955) or use of antiretroviral therapy (10/14 vs 13/18; p=0.981). The rate of BCNIE (Table 2) was significantly lower in the prospective group (28/65 or 43.1%) as compared to the retrospective group (47/75 or 62.7%; p=0.039) The number of patients with BCNIE with no organism or cause detected (NODIE) was significantly lower in the prospective cohort compared to the retrospective

cohort (13.8% vs 57.4%; p<0.01). The in-hospital mortality rate was 23.4% in the retrospective group with BCNIE compared to 14.2% in the prospective cohort (p=0.35).

The baseline comparison of patients with BCPIE and BCNIE in the prospective cohort is summarized in Table 3. The baseline characteristics of these groups were similar, except for the number of intravenous (IV) drug abusers that was significantly higher in the BCPIE group (5 vs 0) and the number of current smokers that was significantly higher in the BCNIE group. (27% vs 60.3%; p=0.002) The rate of antibiotic use prior to blood culture sampling was not significantly different in the BCPIE group when compared to the BCNIE group (19.4 vs 35.7%; p=0.15).

Serology for *Bartonella* and *Mycoplasma* species (15/28; 53.5%) and heart valve PCR (9/20; 45%) had the highest yield for identifying the causative organism in patients with BCNIE (Table 4).

The most common causes of BCPIE (Figure 2) in the prospective cohort were *Staphylococcus aureus* (45.9%) and the viridans group of streptococci (27%). This causes of BCPIE were similar in the retrospective cohort with *Staphylococcus aureus* (43%) and the viridans group of streptococci (32%) the most common. The most common cause of BCNIE (Figure 3) in the prospective cohort was *Bartonella* species (46%).

Considering the comprehensive microbiological evaluation, including serology and PCR data, a causative organism was identified in 86.2% of patients (Figure 3) in the prospective cohort, with *Staphylococcus aureus* (26.2%), *Bartonella* species (20%) and the viridans group of streptococci (15.3%) being the most common.

Discussion

The establishment of a set protocol for organism detection has significantly decreased the number of patients with IE where no causative organism or disease is detected (NODIE). This has been achieved by a significant reduction in the incidence of BCNIE and an improvement in non-culture identification of *Bartonella* species (*Bartonella quintana* in particular), *Mycoplasma* species and *Mycobacterium tuberculosis* as causes of BCNIE in the Western Cape region of South Africa. The finding of *Bartonella* species as the most common cause of BCNIE contrasts with European data where *Coxiella burnettii* has been demonstrated to be the most common cause of BCNIE.(2,6,9,16) No previous study has systematically evaluated the causes of BCNIE in South Africa. However, evaluation of patients with BCNIE in Algeria and Ethiopia, developing nations similar to South Africa, also found *Bartonella* species to be the commonest cause of BCNIE.(2,9,11). This finding has important implications for future diagnostic algorithms and empirical therapy in South Africa. Current guideline empirical therapy for IE has limited efficacy against *Bartonella* and *Mycoplasma* species, this would suggest that a significant number of BCNIE patients may previously have been inadequately treated.(2) In this relatively small cohort of BCNIE patients, we demonstrated a 39.3% reduction in in-hospital mortality (23.4% vs 14.2%; p=0.35). This reduction is likely due to the introduction of an Endocarditis team (2) and the increased detection and subsequent effective treatment of the causative organism. A variety of factors may have contributed to the fact that this did not reach statistical significance. Our protocol dictated that we only perform additional investigations if initial blood cultures remained negative. This meant that additional investigations were only done five days after presentation and the addition of appropriate antibiotic therapy in patients with *Bartonella* species and other fastidious organisms were necessarily delayed beyond five days. During the Covid-19 pandemic, strain on health care resources also caused some delay in surgical

intervention and the performing of blood and heart valve PCR. We would propose that serology for both *Bartonella* and *Mycoplasma* species be performed as part of the initial work up of patients with suspected IE. The addition of doxycycline to current guideline empirical therapy for BCNIE in countries with known or likely high rates of these organisms, should be considered. (2,17,18) Doxycycline has proven effective in the treatment of both Bartonella-and-Mycoplasma associated IE, although current guidelines propose levofloxacin as first line therapy for Mycoplasma associated IE.(2,18) The availability, low cost and favourable side effect profile of doxycycline makes it an ideal add on therapy in South Africa.(19)

This is the first report of the effect that a set protocol for organism detection, managed by an Endocarditis Team, has on the incidence of BCNIE in South Africa and it mimics the reduction reported from other groups.(8) Although the rate of antibiotic administration prior to blood culture sampling was still high (25.6%), the introduction of an Endocarditis Team managed to reduce the rate of antibiotic use prior to blood culture sampling compared to a previous prospective study at our institution (25.6% vs 52.2%).(8) More specific data regarding antibiotic use prior to blood culture sampling was unfortunately not available for the retrospective cohort. This effect of the Endocarditis Team may be due to increased awareness and upskilling of the initial treating physicians as well as improving pathways for referral and further management.(20) The reduction in antibiotic use prior to blood culture sampling was an important contributor to the decrease in BCNIE patients in the prospective cohort compared to the retrospective cohort (61.3%; p=0.039) and previous prospective cohort study (55.3%) performed at our institution.(8,12) Additional factors that may have contributed to the lowering of the BCNIE rate was the mandatory collection of a minimum of

3 sets of blood cultures, repeated sampling if clinical features of infection persist and the routine use of both aerobic and anaerobic blood culture bottles.

The spectrum of BCPIE has changed in South Africa, with a change to a profile similar to developed countries. In both our retrospective and prospective cohorts *Staphylococcus aureus* was the most common causative organism, which contrasts with a previous series from our centre.(8) The demographic profile of patients in both our cohorts were similar to previous series (8), except for the significant increase in intravenous (IV) drug users. All patients who volunteered an IV drug use history were culture positive for *Staphylococcus aureus* (10/10; 100%). However, even if IV drug users were excluded, *Staphylococcus aureus* remained the most common causative organism in both cohorts. Some empirical protocols for the treatment of IE in South Africa still exclude specific *Staphylococcus aureus* targeted antimicrobials (no addition of cloxacillin) because of previous data demonstrating the viridans group of streptococci to be the most common cause of IE with low rates of *Staphylococcus aureus* associated IE.(1,8) Our data strongly support the empirical use of antimicrobial drugs that specifically target *Staphylococcus aureus,* as this is now established as the most common cause of IE in South Africa.

The different additional investigations to identify causes of BCNIE yielded contrasting results. Serology (53.5%) and heart valve PCR (45%) had the highest yield for identifying causes of BCNIE. Of the 13 patients with serological evidence of active Bartonella infection (21) in the setting of BCNIE, eight patients underwent surgery. Heart valve PCR was positive in 7 of the 8 patients (88%); confirming *Bartonella quintana* (6 patients) *and Bartonella henselae* (1 patient) as the causative organism. This would suggest that serology for *Bartonella* species should be added to the initial venous blood analysis of all patients with suspected IE in South Africa. In addition, all patients with BCNIE undergoing surgery should

have heart valve PCR performed in addition to culture, as the yield for Bartonella is much higher with heart valve PCR.

Histological evaluation, especially microscopy, is an important additional investigation in patients undergoing valve surgery. Histopathology confirmed endocarditis in all of the patients in whom surgery was performed and confirmed *Mycobacterium tuberculosis* associated IE in 1 patient with clinical and imaging features typical of *Mycobacterium tuberculosis* associated IE.(1) The diagnosis of tuberculous IE is usually suspected on typical clinical and imaging findings (1) and confirmed with histopathology. Microbiological confirmation remains difficult.(2) A second patient was diagnosed with *Mycobacterium tuberculosis* associated IE on the basis of typical clinical and imaging findings combined with a positive urinary lipoarabinomannan (u-LAM) test. Histopathology revealed typical features of IE, but no typical features of *Mycobacterium tuberculosis* associated IE. This patient had been treated with anti-tuberculous antimicrobials for 31 days before surgery and this might have contributed to the inability of histopathology to identify *Mycobacterium tuberculosis* as the causative organism.

Both patients with Mycobacterium tuberculosis associated IE returned negative Mycobacterium specific (BACTEC Myco/F Lytic) blood cultures; none of the Mycobacterium-specific blood cultures was positive in any of the patients in the BCNIE group.

Blood PCR for patients with BCNIE had a very low yield (4.5%), with only a single positive result. The organism (*Mycoplasma hominis*) was also detected with heart valve PCR putting into question whether blood PCR is a cost-effective test for patients with BCNIE. Although low positive serology for *Coxiella burnetii*, *Legionella pneumonia* and *Brucella* species were common, not a single patient fulfilled criteria for regarding these as the causative organism.(2) One patient with Systemic Lupus Erythematosus (SLE) returned positive

serology for Brucella and Bartonella species; on final analysis these were considered false positive due to cross reactivity.

Conclusion

The introduction of a set protocol for organism detection with diagnosis and management by an Endocarditis Team not only lowered the rate of BCNIE but detected causative organisms that are difficult or impossible to culture. *Bartonella* species, and *Bartonella quintana* in particular, is the most common cause of BCNIE in the Western Cape, South Africa and empirical therapy directed at *Bartonella* species should be considered in patients with BCNIE. Future trials should evaluate if early therapy directed at *Bartonella* species as part of empirical therapy for IE improve short- and long-term outcomes. *Staphylococcus aureus* has now been established as the most common cause of BCPIE in South Africa and all empirical regiments should include specific anti-staphylococcal therapy.

Limitations

The causative organisms associated with IE, in particular BCNIE, varies according to geographic region.(1,7) This may limit the generalisability of our results.

During a large part of the study, the Covid-19 pandemic had a significant influence on health care services in South Africa.(22–24) The exact influence of this pandemic on the treatment of IE in our institution is difficult to quantify, but it is safe to assume that delays from diagnosis to surgery were contributed to by the pandemic. The fact that we could still demonstrate a reduction in in-hospital mortality, although not statistically significant, strengthens the argument that the introduction of an Endocarditis team with a set protocol for organism detection should improve patient outcomes.

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Availability of data and material: All data is securely stored on a digital database that is password protected. Data is available for review on reasonable request.

Contributorship statement:

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript. Alfonso Pecoraro as the primary investigator was responsible for the conception and design of the study, acquisition of data, analysis and interpretation as well as drafting of the manuscript.

Colette Pienaar, Philippus Herbst and Anton Doubell contributed to the conception and design of the study, acquisition of data, analysis and interpretation as well as revising the manuscript critically for important intellectual content.

Simon Poerstamper, Lloyd Joubert, Hans Prozesky, Jantjie Taljaard, Jacques Janson and Mae Newton-Foot contributed to the acquisition, analysis and interpretation of data as well as revising the manuscript critically for important intellectual content.

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Table 1: Demographic profile

	All patients n=140	Retrospective cohort n=75	Prospective cohort n=65	p-value
Age in years, mean (SD)	39,6 (12.8)	39,6(12.4)	39,5(13.1)	0,80
Male sex	94 (67.1%)	51 (68%)	43 (67%)	0,81
Diabetes	7 (5%)	3 (4%)	4 (6.3%)	0,55
Hypertension	25 (17.9%)	15 (20%)	10 (15.6%)	0,51
Current smokers	51 (34.4%)	27 (36%)	24 (37.5%)	0,857
HIV positive	32 (22.9%)	14 (21.5%)	18 (29%)	0,21
CD4 count in cells/µL, median (Q1;Q3)	423	442 (137;568)	409 (204;568)	0,955
Current ARV* therapy	23 (71.8%)	10/14 ((71.4%)	13/18 (72%)	0,981
History of IV drug abuse	10 (7.1%)	5 (6.7%)	5 (7.7%)	0,925
History of valvular heart disease	40 (28.6%)	24 (32%)	16 (25%)	0,367
Previous cardiac surgery	20 (14.3%)	10 (13.3%)	10 (15.6%)	0,705

**Anti-retroviral treatment*

Table 2: Results of blood cultures and short term mortality

	All patients n=140	Retrospective cohort n=75	Prospective cohort n=65	p-value
BCNIE	75 (53.6%)	47 (62,7%)	28 (43,1%)	0,020
BCNIE with no organism or cause detected (NODIE)	53 (37.9%)	44 (57.4%)	9 (13.8%)	<0,01
In hospital mortality BCPIE	10/65 (15.4%)	5/28 (17.9%)	5/37 (13.5%)	0,64
In hospital mortality BCNIE	15/75 (20%)	11/47(23.4%)	4/28 (14.2%)	0,35

Table 3 Baseline characteristics of BCPIE vs BCNIE in the prospective cohort

	BCPIE (n=37)	BCNIE	P

		(n=28)	value
Age (mean)	39,75	38,2	0,64
Male sex	23 (63.9%)	20 (71.4%)	0,44
Diabetes	4 (11.1%)	0	
Hypertension	5 (13.9%)	5 (17.9%)	0,68
Current smokers	7 (27%)	17 (60,7%)	0,002
HIV positive	9 (25.7%)	9 (33.3%)	0,49
CD4 count cells/µL (mean)	347	470	0,67
Current ARV* therapy	6 (66.7%)	7 (77.8%)	0.65
History of IV drug abuse	5 (13.5%)	0	
History of valvular heart disease	9 (25%)	7 (25%)	1
Previous cardiac surgery	7 (19.4%)	3 (10.7%)	0,35
Antibiotic therapy prior to blood culture sampling	7 (19.4%)	10 (35.7%)	0,15
Surgery performed	19	20	0,11
In hospital mortality	5 (13.5%)	4 (14.2%)	0,949

**Anti-retroviral treatment*

Table 4: Results of set protocol for organism detection in patients with BCNIE in the prospective cohort

Test performed	n=28
Mycobacterium specific blood cultures positive	0
Anti-nuclear antibodies positive (ANA)	1
Serology indicating acute infection	15 (53.5%)
• *Bartonella species*	13
• *Mycoplasma species*	2
Blood PCR positive	1/22 (4.5%)
• Mycoplasma hominis	1
Heart valve PCR positive	9/20 (45%)
• *Bartonella quintana*	6
• *Bartonella henselae*	1

• *Mycoplasma hominis*	1
• *Alternaria species*	1
Histopathological confirmed IE	20/20
• Cause identified (*Mycobacterium tuberculosis*)	1/20
Heart valve culture positive	0 / 20

Figure 1: Protocol for organism detection

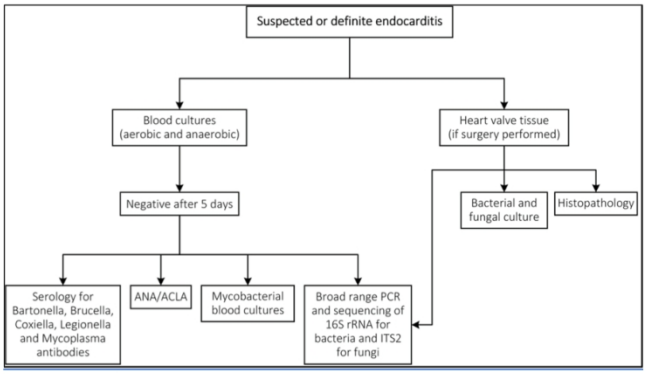
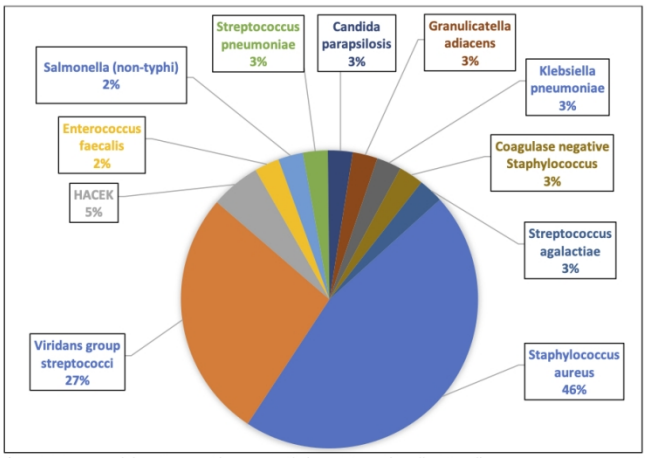


Figure 1: Protocol for organism detection

419x593mm (72 x 72 DPI)

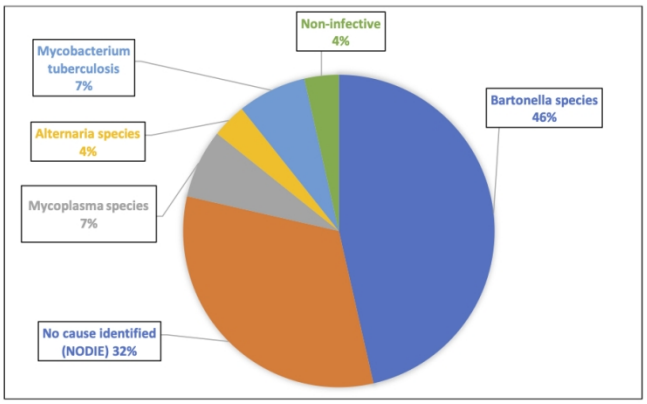
Figure 2: Causes of BCPIE (%)



*HACEK - Haemophilus, Aggregatibacter, Cardiobacterium, Eikenella, Kingella

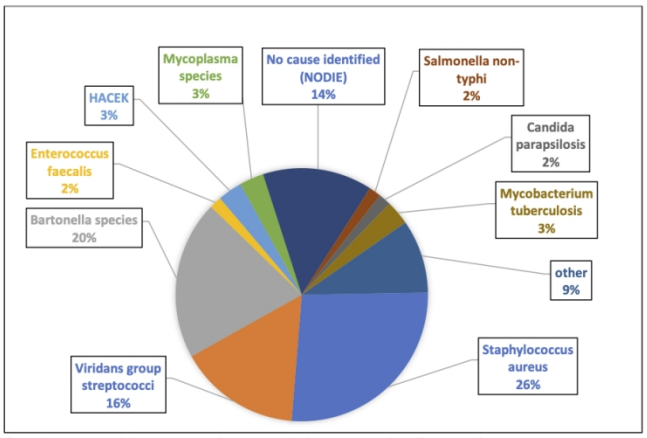
419x594mm (72 x 72 DPI)

Figure 3: Causes of BCNIE (%) detected by non-culture dependent techniques



419x594mm (72 x 72 DPI)

Figure 4: Causes of IE after set protocol for organism detected



*HACEK - Haemophilus, Aggregatibacter, Cardiobacterium, Eikenella, Kingella

419x594mm (72 x 72 DPI)

Addendum A: Stepwise approach to identify causative organism or non-infective cause

A stepwise approach was employed to identify the causative organisms of IE and to minimise the incidence of BCNIE. At least three sets of blood cultures per patient were collected using an aseptic technique. Each set was drawn from a different peripheral site and included a BacT/ALERT® FA Plus (aerobic) (bioMérieux, Marcy l'Étoile, France) and BacT/ALERT® FN Plus (anaerobic) bottle. Additional blood cultures were obtained if clinical features of infection persisted. The blood cultures were submitted to the Microbiology laboratory of the National Health Laboratory Service (NHLS) at Tygerberg Hospital, and incubated in the BacT/ALERT® 3D automated microbial detection system for five days. Once the instrument detected growth in the bottles and signalled them as positive, they were removed, a Gram stain performed and the clinician immediately phoned and informed of the result. Depending on the organism observed on Gram stain, the blood culture broth was sub-cultured onto appropriate solid culture media such as blood agar, chocolate agar and MacConkey agar (prepared in-house), followed by incubation at the required temperature and atmosphere to optimise growth. The agar plates were examined after 18-24 hours of incubation and isolates identified using manual and/or automated biochemical assays; if these methods failed, isolates were referred for mass spectrometry (VITEK® MS, bioMérieux, Marcy l'Étoile, France). Antimicrobial susceptibility testing (AST) was performed by disk diffusion, automated broth dilution (VITEK® 2, bioMérieux, Marcy l'Étoile, France) and/or Etest® (bioMérieux, Marcy l'Étoile, France). AST results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) criteria. If no organism was isolated after five days using standard culture techniques, patients were defined as having BCNIE. Further testing performed on these patients are summarised in Figure 1 and included:

- Serology: Indirect immunofluorescence assays (IFA) for detection of antibodies to *Bartonella henselae* and *B. quintana* (Bartonella IFA IgM and IgG kits, FOCUS Diagnostics, Cypress, CA, USA), and *C. burnetii* (Q Fever IgM and IgG kits, FOCUS Diagnostics, Cypress, CA, USA). Enzyme-linked immunosorbent assays (ELISA) were performed to detect IgM and IgG antibodies to *Brucella* species (MASTAZYME BRUCELLA kit, MAST DIAGNOSTICA, Reinfeld, Germany), *Legionella pneumophila* (EUROIMMUN, Lübeck, Germany) and *Mycoplasma pneumoniae* (EUROIMMUN, Lübeck, Germany).
- Auto-antibody testing: Antinuclear antibodies (ANA) were detected with the Kallestad® HEp-2 Cell Line Substrate and Kallestad Mouse Stomach/Kidney Test kits (Bio-Rad Laboratories Inc, Redmond, WA, USA), and anti-cardiolipin antibodies (ACLA) with the QUANTA Lite PR3 SC and MPO SC ELISA kits (Inova Diagnostics, San Diego, CA, USA).

- Molecular testing on negative blood cultures: DNA was extracted from 200 µl of blood culture broth using the tissue protocol of the Qiagen QIAamp DNA Mini Kit, following benzyl alcohol extraction, as previously described. (1) Bacterial 16S rRNA PCR amplification was performed using primers BAK11w and BAK2 (2) and KAPA2G Robust HotStart ReadyMix (Kapa Biosystems, South Africa) according to manufacturer's instructions, with an annealing temperature of 55°C for 30 cycles. Fungal ITS2 amplification was performed using primers ITS1 and ITS4 (3) and KAPA Taq ReadyMix (Kapa Biosystems) in a touchdown PCR with annealing temperatures of 56°C for 10 cycles and 54°C for 30 cycles. Amplicons were sequenced on the ABI 3500XL genetic analyser at Inqaba Biotec (South Africa). Bacterial and fungal identification was based on >99% sequence alignment to published sequences available in the National Center for Biotechnology Information's Genbank database.
- Mycobacterial blood cultures: BACTEC™ Myco/F Lytic Culture vials (Becton Dickinson, Sparks, MD, USA) were collected for the isolation of *Mycobacterium tuberculosis* (MTB) and non-tuberculous Mycobacteria. The bottles were incubated in an automated continuous monitoring BACTEC 9120 instrument for 42 days. Work-up of positive cultures is not included since all cultures were negative.

If surgery was performed, heart valve tissue was submitted for:

- Bacterial and fungal culture
- Broad range PCR and sequencing of 16S rRNA for bacteria and ITS2 for fungi
- Histopathologic examination for detection of bacteria and fungi, as well as histopathological features of IE.

References:

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STROBE Statement— Causes of infective endocarditis in the Western Cape, South Africa

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	2
Objectives	3	State specific objectives, including any prespecified hypotheses	2
Methods			
Study design	4	Present key elements of study design early in the paper	3
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	3
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	3
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	3
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*4*
Bias	9	Describe any efforts to address potential sources of bias	4
Study size	10	Explain how the study size was arrived at	n/a
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	4
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	4
		(*b*) Describe any methods used to examine subgroups and interactions	4
		(*c*) Explain how missing data were addressed	n/a
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	n/a
		(*e*) Describe any sensitivity analyses	

Continued on next page

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	5
		(b) Give reasons for non-participation at each stage	n/a
		(c) Consider use of a flow diagram	n/a
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	5
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	*5*
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	5
		(*b*) Report category boundaries when continuous variables were categorized	n/a
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n/a
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	5
Discussion			
Key results	18	Summarise key results with reference to study objectives	6-8
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	8
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	8
Generalisability	21	Discuss the generalisability (external validity) of the study results	8-9
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	9

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Causes of infective endocarditis in the Western Cape, South Africa: A prospective cohort study utilising a set protocol for organism detection and central decision making by an Endocarditis team

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Primary Subject Heading:	Cardiovascular medicine
Secondary Subject Heading:	Cardiovascular medicine
Keywords:	Adult cardiology < CARDIOLOGY, Valvular heart disease < CARDIOLOGY, Diagnostic microbiology < INFECTIOUS DISEASES, Epidemiology < INFECTIOUS DISEASES, MICROBIOLOGY

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Title page:

Causes of infective endocarditis in the Western Cape, South Africa: A prospective cohort study utilising a set protocol for organism detection and central decision making by an Endocarditis team

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Keywords: Infective endocarditis, Endocarditis Team, blood culture negative infective endocarditis

Word count: 3031

1
2

Abstract

Background:

Blood culture negative infective endocarditis(BCNIE) poses both a diagnostic and therapeutic challenge. High rates of BCNIE reported in South Africa have been attributed to antibiotic use prior to blood culture sampling.

Objectives:

To assess the impact of a systematic approach to organism detection and identify the causes of infective endocarditis, in particular causes of BCNIE.

Design:

Prospective cohort study.

Methods:

The Tygerberg Endocarditis Cohort(TEC) study prospectively enrolled patients with infective endocarditis(IE) between November 2019 and February 2021. A set protocol for organism detection with management of patients by an Endocarditis Team was employed. This prospective cohort was compared to a retrospective cohort of patients with IE admitted between January 2017 and December 2018.

Results:

Hundred and forty patients with IE were included, with 75 and 65 patients in the retrospective and prospective cohorts respectively. Baseline demographic characteristics were similar with a mean age of 39,6 years and male predominance(male sex = 67.1) The rate of BCNIE was lower in the prospective group(28/65 or 43.1%) compared to the retrospective group(47/75 or 62.7%;p=0.039). The BCNIE in-hospital mortality rate in the retrospective cohort was 23.4% compared to 14.2% in the prospective cohort(p=0.35). A cause was identified (including non-culture techniques) in 86.2% of patients in the prospective cohort, with *Staphylococcus aureus*(26.2%), *Bartonella* species(20%) and the viridans streptococci(15.3%) being most common.

Conclusion:

The introduction of a set protocol for organism detection, managed by an Endocarditis Team, has identified *Staphylococcus aureus* as the most common cause of IE and identified non-culturable organisms, in particular *Bartonella quintana*, as an important cause of BCNIE. A reduction in in-hospital mortality in patients with BCNIE was observed, but did not reach statistical significance.

Strengths and limitations of this study

- This is the first prospective cohort study that has evaluated the impact of an Endocarditis Team, with a set protocol for organism detection, on patients with IE in South Africa
- A comprehensive protocol for organism detection was employed to minimize the rate of BCNIE and identify non-culturable organisms
- Causative organisms of IE, in particular BCNIE, varies geographically. This may limit the generalisability of this data

Introduction

Infective endocarditis (IE) is an infection involving the endocardial surface of the heart. This can affect native heart valves (native valve endocarditis or NVE), prosthetic valves (prosthetic valve endocarditis or PVE), non-valvular endocardial surfaces (such as IE affecting ventricular septal defects) or any non-valvular prosthetic devices.(1–4) Identification of the causative organism via blood cultures is fundamental to the diagnosis and treatment of IE.(2,4) Blood cultures that fail to identify the causative organism in patients with clinical and / or imaging evidence of IE - so called blood culture negative IE (BCNIE) - pose both a diagnostic and therapeutic challenge to the treating physician. BCNIE has been associated with worse outcomes compared to patients with blood culture positive IE (BCPIE), although more recent reports have demonstrated equivalent

outcomes.(5–8) It is important to note that the proportion of patients with BCNIE has decreased, which is likely due to a decrease in antibiotic use prior to blood culture collection.(6,7) Although BCNIE is still diagnosed in a significant proportion of patients with IE, in the majority of patients the organism or cause is identified via non-culture-dependant methods. The identification of organisms responsible for BCNIE (and thus appropriate treatment) has coincided with more equal outcomes when comparing BCNIE to BCPIE presumably due to more targeted therapy.(7,9) BCNIE was previously mainly attributed to sterilized blood cultures due to antibiotic use prior to acquisition of adequate blood culture samples. Although this is still a contributor, IE caused by organisms that are either intracellular or difficult to culture with standard culture methods, have emerged as an important cause of BCNIE.(7,10,11) These organisms vary according to geographic region with *Coxiella burnettii* more common in European cohorts in contrast to African cohorts demonstrating *Bartonella* species as the most common cause of BCNIE.(8,10,12) Non-infectious causes e.g. non-bacterial thrombotic endocarditis are rare causes of BCNIE.(9,13,14)

Very high rates of BCNIE have been reported in South Africa varying from 40-65%.(9,13,14) This was attributed to high rates of antibiotic use prior to blood culture sampling (25%-52%), although no systematic approach to organism detection was employed and thus no information is available about the other causes of BCNIE in South Africa.(2,9,14) Our group recently reported the emergence and typical clinical and imaging findings of *Bartonella* species as a cause of BCNIE in South Africa.(15)

We postulated that the implementation of a set protocol for organism detection and management of patients with IE by an Endocarditis Team would identify causes of BCNIE and improve the short-term outcome of patients with both BCPIE and BCNIE.

Methods

All patients presenting to the Division of Cardiology, Department of Medicine at Tygerberg Hospital in Cape Town, South Africa, with IE by current criteria (2) between November 2019 and February 2021 were prospectively included in the Tygerberg Endocarditis Cohort (TEC) study. Patients with known or newly diagnosed malignancy were excluded from this study. The Division of Cardiology at Tygerberg Hospital is a public sector tertiary referral centre that serves a population of approximately 2.4 million people.(16) All patients presenting with features of IE to hospitals within the referral network are referred to Tygerberg Hospital for definitive care.

All patients were managed by an Endocarditis Team (2) which fulfilled all the criteria as set out by current guidelines. All patients underwent standard transthoracic echocardiography (TTE) and transoesophageal echocardiography (TEE) in the absence of identifiable contra-indications to TEE. Additional imaging was performed at the discretion of the Endocarditis Team.

A stepwise protocol for organism detection (supplementary file A) was utilised to identify the causative organisms of IE and to minimize the incidence of BCNIE (Figure 1). A minimum of three sets of blood cultures (BacT/ALERT, bioMérieux, Marcy l'Etoile, France), including one aerobic and one anaerobic bottle per set, were required, with repeated cultures if clinical features of infection persisted. Further management and analysis of the samples were done according to current published guidelines.(2,15) Patients without an identified organism after five days, using standard culture techniques, were defined as BCNIE.

All BCNIE patients underwent venous blood analysis (all test performed in parallel) for further testing, including:

- Testing for antinuclear antibodies (ANA) and anti-cardiolipin antibodies (ACLA)
- Serology was performed using indirect immunofluorescence assays (IFA) for detection of IgM and IgG antibodies to *Bartonella henselae* and *Bartonella quintana* (FOCUS Diagnostics, Cypress, CA, USA). Specific antibodies to *Coxiella burnetii* were also determined by IFA. Enzyme immunoassays (EIA) were performed to detect IgM and IgG antibodies to *Brucella* species, *Legionella pneumophila* (EUROIMMUN, Lübeck, Germany) and *Mycoplasma pneumoniae* (EUROIMMUN, Lübeck, Germany).
- Direct polymerase chain reaction (PCR) was performed on blood culture bottles for detection of the universal bacterial 16S rRNA and ITS2 for fungi, followed by sequencing to identify the amplified DNA product
- BACTEC Myco/F Lytic vials (Becton Dickinson, San Jose, CA, USA) were collected for the isolation of Mycobacteria, including *Mycobacterium tuberculosis* (MTB) and non-tuberculous Mycobacteria

A sample of heart valve tissue was collected from all patients who required surgery and this was submitted for:

- Bacterial and fungal culture
- Broad range PCR with 16S rRNA for bacteria and ITS2 for fungi, followed by sequencing to identify the amplified DNA product
- Histopathologic examination to detect bacteria and fungi, as well as histopathological features of IE

All patients were managed according to current guidelines by the Endocarditis Team and prospectively followed.(2,15) Baseline demographic and clinical features, results of special investigations including microbiological evaluation and imaging findings were documented on all patients. Treatment strategy, including specific antimicrobial therapy and surgical interventions were documented. Patients were followed till hospital discharge and all major adverse events (death, embolic events, renal failure) were recorded.

To evaluate the impact of this strategy, the prospective cohort was compared with a retrospective cohort that comprised of patients with IE admitted to Tygerberg Hospital from January 2017 to December 2018. In this latter cohort, diagnostic evaluation and treatment was not standardized and at the discretion of the managing physician (rather than formalised in an Endocarditis Team) and without a step wise protocol for organism detection. Serology, blood rRNA PCR on blood and heart valve PCR was rarely performed. All retrospective data was collected from patient folders, echocardiography-, laboratory- and surgical databases.

Patients who presented within the Tygerberg Hospital referral network but surmised due to IE before referral to Tygerberg Hospital was included to minimise selection bias.

Statistical analysis

Statistical analysis was done using SPSS v27 for iOS and JASP (Version 0.14.1) for iOS. Descriptive statistics were calculated, nominal data was compared via cross tabulation and Chi-square tests, parametric data was compared using independent-sample T-tests (Cohen's d) and non-parametric data was compared using independent-samples T-test (Mann-Whitney U or Kruskal-Wallis 1-way ANOVA).

Ethical considerations

This study was approved by the Health Research Ethics Committee (HREC) of Stellenbosch University (Project numbers S19/08/162 and S19/10/234) and performed in accordance with the Declaration of Helsinki (2013 version). All patients in the prospective cohort signed written informed consent; a waiver of consent was obtained from HREC to include patients in the retrospective cohort.

Patient and public involvement

It was not possible to involve patients or the public in the design, or conduct, or reporting, or dissemination plans of our research.

Results

A total of 140 patients with IE were included, with 75 and 65 patients in the retrospective and prospective cohorts respectively. The baseline characteristics of patients in both cohorts are summarized in Table 1. The mean age was 39,6 years with a male predominance (male sex = 67.1%). Fourteen of the 75 patients (21.5%) in the retrospective cohort were HIV-positive compared to 18 of 65 (29%) in the prospective cohort (p=0.21). There was no difference in absolute CD4 count (442cells/µl vs 402cells/µl; p=0.96) or use of antiretroviral therapy (10/14 vs 13/18; p=0.98). The rate of BCNIE (Table 2) was significantly lower in the prospective group (28/65 or 43.1%) as compared to the retrospective group (47/75 or 62.7%; p=0.04) The number of patients with BCNIE with no organism or cause detected was significantly lower in the prospective cohort compared to the retrospective cohort (13.8% vs 57.4%; p<0.01). The in-hospital mortality rate was 23.4% in the retrospective group with BCNIE compared to 14.2% in the prospective cohort (p=0.35).

The baseline comparison of patients with BCPIE and BCNIE in the prospective cohort is summarized in Table 3. The baseline characteristics of these groups were similar, except for the number of intravenous (IV) drug abusers that was significantly higher in the BCPIE group (5 vs 0) and the number of current smokers that was significantly higher in the BCNIE group. (27% vs 60.3%; p<0.01) The rate of antibiotic use prior to blood culture sampling was not significantly different in the BCPIE group when compared to the BCNIE group (19.4 vs 35.7%; p=0.15).

Serology for *Bartonella* and *Mycoplasma* species (15/28; 53.5%) and heart valve PCR (9/20; 45%) had the highest yield for identifying the causative organism in patients with BCNIE (Table 4).

The most common causes of BCPIE (Figure 2) in the prospective cohort were *Staphylococcus aureus* (45.9%) and the viridans streptococci (27%). This causes of BCPIE were similar in the retrospective cohort with *Staphylococcus aureus* (43%) and the viridans streptococci (32%) the most common. The most common cause of BCNIE (Figure 3) in the prospective cohort was *Bartonella* species (46%).

Considering the comprehensive microbiological evaluation, including serology and PCR data, a causative organism was identified in 86.2% of patients (Figure 4) in the prospective cohort, with *Staphylococcus aureus* (26.2%), *Bartonella* species (20%) and the viridans streptococci (15.3%) being the most common.

Discussion

The establishment of a set protocol for organism detection has significantly decreased the number of patients with IE where no causative organism or disease is detected. This has been achieved by a significant reduction in the incidence of BCNIE and an improvement in

non-culture identification of *Bartonella* species (*Bartonella quintana* in particular), *Mycoplasma* species and *Mycobacterium tuberculosis* as causes of BCNIE in the Western Cape region of South Africa. The finding of *Bartonella* species as the most common cause of BCNIE contrasts with European data where *Coxiella burnettii* has been demonstrated to be the most common cause of BCNIE.(2,6,10,17) No previous study has systematically evaluated the causes of BCNIE in South Africa. However, evaluation of patients with BCNIE in Algeria and Ethiopia, developing nations similar to South Africa, also found *Bartonella* species to be the commonest cause of BCNIE.(2,10,12). This finding has important implications for future diagnostic algorithms and empirical therapy in South Africa. Current guideline empirical therapy for IE has limited efficacy against *Bartonella* and *Mycoplasma* species, this would suggest that a significant number of BCNIE patients may previously have been inadequately treated.(2) In this relatively small cohort of BCNIE patients, we demonstrated a 39.3% reduction in in-hospital mortality (23.4% vs 14.2%; p=0.35). This reduction may be due to the introduction of an Endocarditis team (2) and the increased detection and subsequent effective treatment of the causative organism. A variety of factors may have contributed to the fact that this did not reach statistical significance. Our protocol dictated that we only perform additional investigations if initial blood cultures remained negative. This meant that additional investigations were only done five days after presentation and the addition of appropriate antibiotic therapy in patients with *Bartonella* species and other fastidious organisms were necessarily delayed beyond five days. During the Covid-19 pandemic, strain on health care resources also caused some delay in surgical intervention and the performing of blood and heart valve PCR. We would propose that serology for both *Bartonella* and *Mycoplasma* species be performed as part of the initial work up of patients with suspected IE. The addition of doxycycline to current guideline

empirical therapy for BCNIE in countries with known or likely high rates of these organisms, should be considered. (2,18,19) Doxycycline has proven effective in the treatment of both Bartonella-and-Mycoplasma associated IE, although current guidelines propose levofloxacin as first line therapy for Mycoplasma associated IE.(2,19) The availability, low cost and favourable side effect profile of doxycycline makes it an ideal add on therapy in South Africa.(20)

This is the first report of the effect that a set protocol for organism detection, managed by an Endocarditis Team, has on the incidence of BCNIE in South Africa and it mimics the reduction reported from other groups.(9) Although the rate of antibiotic administration prior to blood culture sampling was still high (25.6%), the introduction of an Endocarditis Team managed to reduce the rate of antibiotic use prior to blood culture sampling compared to a previous prospective study at our institution (25.6% vs 52.2%).(9) More specific data regarding antibiotic use prior to blood culture sampling was unfortunately not available for the retrospective cohort. This effect of the Endocarditis Team may be due to increased awareness and upskilling of the initial treating physicians as well as improving pathways for referral and further management.(21) The reduction in antibiotic use prior to blood culture sampling was an important contributor to the decrease in BCNIE patients in the prospective cohort compared to the retrospective cohort (61.3%; p=0.039) and previous prospective cohort study (55.3%) performed at our institution.(9,13) Additional factors that may have contributed to the lowering of the BCNIE rate was the mandatory collection of a minimum of 3 sets of blood cultures, repeated sampling if clinical features of infection persist and the routine use of both aerobic and anaerobic blood culture bottles.

The spectrum of BCPIE has changed in South Africa, with a change to a profile similar to developed countries. In both our retrospective and prospective cohorts *Staphylococcus*

aureus was the most common causative organism, which contrasts with a previous series from our centre.(9) The demographic profile of patients in both our cohorts were similar to previous series (9), except for the significant increase in intravenous (IV) drug users. All patients who volunteered an IV drug use history were culture positive for *Staphylococcus aureus* (10/10; 100%). However, even if IV drug users were excluded, *Staphylococcus aureus* remained the most common causative organism in both cohorts. Some empirical protocols for the treatment of IE in South Africa still exclude specific *Staphylococcus aureus* targeted antimicrobials (no addition of cloxacillin) because of previous data demonstrating the viridans streptococci to be the most common cause of IE with low rates of *Staphylococcus aureus* associated IE.(1,9) Our data strongly support the empirical use of antimicrobial drugs that specifically target *Staphylococcus aureus,* as this is now established as the most common cause of IE in South Africa.

The different additional investigations to identify causes of BCNIE yielded contrasting results. Serology (53.5%) and heart valve PCR (45%) had the highest yield for identifying causes of BCNIE. Of the 13 patients with serological evidence of active Bartonella infection (22) in the setting of BCNIE, eight patients underwent surgery. Heart valve PCR was positive in 7 of the 8 patients (88%); confirming *Bartonella quintana* (6 patients) *and Bartonella henselae* (1 patient) as the causative organism. This would suggest that serology for *Bartonella* species should be added to the initial venous blood analysis of all patients with suspected IE in South Africa. In addition, all patients with BCNIE undergoing surgery should have heart valve PCR performed in addition to culture, as the yield for Bartonella is much higher with heart valve PCR.

Histological evaluation, especially microscopy, is an important additional investigation in patients undergoing valve surgery. Histopathology confirmed endocarditis in all of the

patients in whom surgery was performed and confirmed *Mycobacterium tuberculosis* associated IE in 1 patient with clinical and imaging features typical of *Mycobacterium tuberculosis* associated IE.(1) The diagnosis of tuberculous IE is usually suspected on typical clinical and imaging findings (1) and confirmed with histopathology. Microbiological confirmation remains difficult.(2) A second patient was diagnosed with *Mycobacterium tuberculosis* associated IE on the basis of typical clinical and imaging findings combined with a positive urinary lipoarabinomannan (u-LAM) test. Histopathology revealed typical features of IE, but no typical features of *Mycobacterium tuberculosis* associated IE. This patient had been treated with anti-tuberculous antimicrobials for 31 days before surgery and this might have contributed to the inability of histopathology to identify *Mycobacterium tuberculosis* as the causative organism.

Both patients with Mycobacterium tuberculosis associated IE returned negative Mycobacterium specific (BACTEC Myco/F Lytic) blood cultures; none of the Mycobacterium-specific blood cultures was positive in any of the patients in the BCNIE group.

Blood PCR for patients with BCNIE had a very low yield (4.5%), with only a single positive result. The organism (*Mycoplasma hominis*) was also detected with heart valve PCR putting into question whether blood PCR is a cost-effective test for patients with BCNIE. Although low positive serology for *Coxiella burnetii*, *Legionella pneumonia* and *Brucella* species were common, not a single patient fulfilled criteria for regarding these as the causative organism.(2) One patient with Systemic Lupus Erythematosus (SLE) returned positive serology for Brucella and Bartonella species; on final analysis these were considered false positive due to cross reactivity.

Conclusion

The introduction of a set protocol for organism detection with diagnosis and management by an Endocarditis Team not only lowered the rate of BCNIE but detected causative organisms that are difficult or impossible to culture. *Bartonella* species, and *Bartonella quintana* in particular, is the most common cause of BCNIE in the Western Cape, South Africa and empirical therapy directed at *Bartonella* species should be considered in patients with BCNIE. Future trials should evaluate if early therapy directed at *Bartonella* species as part of empirical therapy for IE improve short- and long-term outcomes. *Staphylococcus aureus* has now been established as the most common cause of BCPIE in South Africa and all empirical regiments should include specific anti-staphylococcal therapy.

Limitations

The causative organisms associated with IE, in particular BCNIE, varies according to geographic region.(1,7) This may limit the generalisability of our results.

During a large part of the study, the Covid-19 pandemic had a significant influence on health care services in South Africa.(23–25) The exact influence of this pandemic on the treatment of IE in our institution is difficult to quantify, but it is safe to assume that delays from diagnosis to surgery were contributed to by the pandemic. The fact that we could still demonstrate a reduction in in-hospital mortality, although not statistically significant, strengthens the argument that the introduction of an Endocarditis team with a set protocol for organism detection should improve patient outcomes. The inability of this study to demonstrate a statistically significant in-hospital mortality benefit is likely due to the small sample size and thus type II statistical error.

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Consent for publication: The authors consent to publication of the data if accepted. Patients consented to the publication of the data and images.

Availability of data and material: All data is securely stored on a digital database that is password protected. Data is available for review on reasonable request.

Contributorship statement:

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript. Alfonso Pecoraro as the primary investigator was responsible for the conception and design of the study, acquisition of data, analysis and interpretation as well as drafting of the manuscript.

Colette Pienaar, Philippus Herbst and Anton Doubell contributed to the conception and design of the study, acquisition of data, analysis and interpretation as well as revising the manuscript critically for important intellectual content.

Simon Poerstamper, Lloyd Joubert, Hans Prozesky, Jantjie Taljaard, Jacques Janson and Mae Newton-Foot contributed to the acquisition, analysis and interpretation of data as well as revising the manuscript critically for important intellectual content.

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Table 1: Demographic profile

	All patients n=140	Retrospective cohort n=75	Prospective cohort n=65	p-value
Age in years, mean (SD)	39.6 (12.8)	39.6(12.4)	39.5(13.1)	0.80
Male sex	94 (67.1%)	51 (68%)	43 (67%)	0.81
Diabetes	7 (5%)	3 (4%)	4 (6.3%)	0.55
Hypertension	25 (17.9%)	15 (20%)	10 (15.6%)	0.51
Current smokers	51 (34.4%)	27 (36%)	24 (37.5%)	0.88
PLHIV	32 (22.9%)	14 (21.5%)	18 (29%)	0,21
CD4 count in cells/µL, median (Q1;Q3)	423	442 (137;568)	409 (204;568)	0.96
c-ART	23 (71.8%)	10/14 ((71.4%)	13/18 (72%)	0.98
History of intravenous drug use	10 (7.1%)	5 (6.7%)	5 (7.7%)	0.93
History of valvular heart disease	40 (28.6%)	24 (32%)	16 (25%)	0.37
Previous cardiac surgery	20 (14.3%)	10 (13.3%)	10 (15.6%)	0.70
Definite IE by the modified Duke/ESC 2015 clinical criteria (2)	83 (59.3%)	35 (46.7%)	48 (73.8%)	<0.01

PLHIV – People living with Human Immunodeficiency Virus; c-ART – combination anti-retroviral therapy

Table 2: Results of blood cultures and short term mortality

	All patients n=140	Retrospective cohort n=75	Prospective cohort n=65	p-value
BCNIE	75 (53.6%)	47 (62.7%)	28 (43.1%)	0.02
BCNIE with no organism or cause detected	53 (37.9%)	44 (57.4%)	9 (13.8%)	<0.01
In hospital mortality BCPIE	10/65 (15.4%)	5/28 (17.9%)	5/37 (13.5%)	0.64
In hospital mortality BCNIE	15/75 (20%)	11/47(23.4%)	4/28 (14.2%)	0.35

BCPIE – Blood culture positive infective endocarditis; BCNIE – Blood culture negative infective endocarditis

Table 3 Baseline characteristics of BCPIE vs BCNIE in the prospective cohort

	BCPIE (n=37)	BCNIE (n=28)	p- value
Age (mean)	39.75	38.2	0.64
Male sex	23 (63.9%)	20 (71.4%)	0.44
Diabetes	4 (11.1%)	0	
Hypertension	5 (13.9%)	5 (17.9%)	0.68
Current smokers	7 (27%)	17 (60.7%)	<0.01
PLHIV	9 (25.7%)	9 (33.3%)	0.49
CD4 count cells/μL (mean)	347	470	0.67
c-ART	6 (66.7%)	7 (77.8%)	0.65
History of intravenous drug use	5 (13.5%)	0	
History of valvular heart disease	9 (25%)	7 (25%)	1
Previous cardiac surgery	7 (19.4%)	3 (10.7%)	0.35
Antibiotic therapy prior to blood culture sampling	7 (19.4%)	10 (35.7%)	0.15
Surgery performed	19	20	0.11
In hospital mortality	5 (13.5%)	4 (14.2%)	0.95

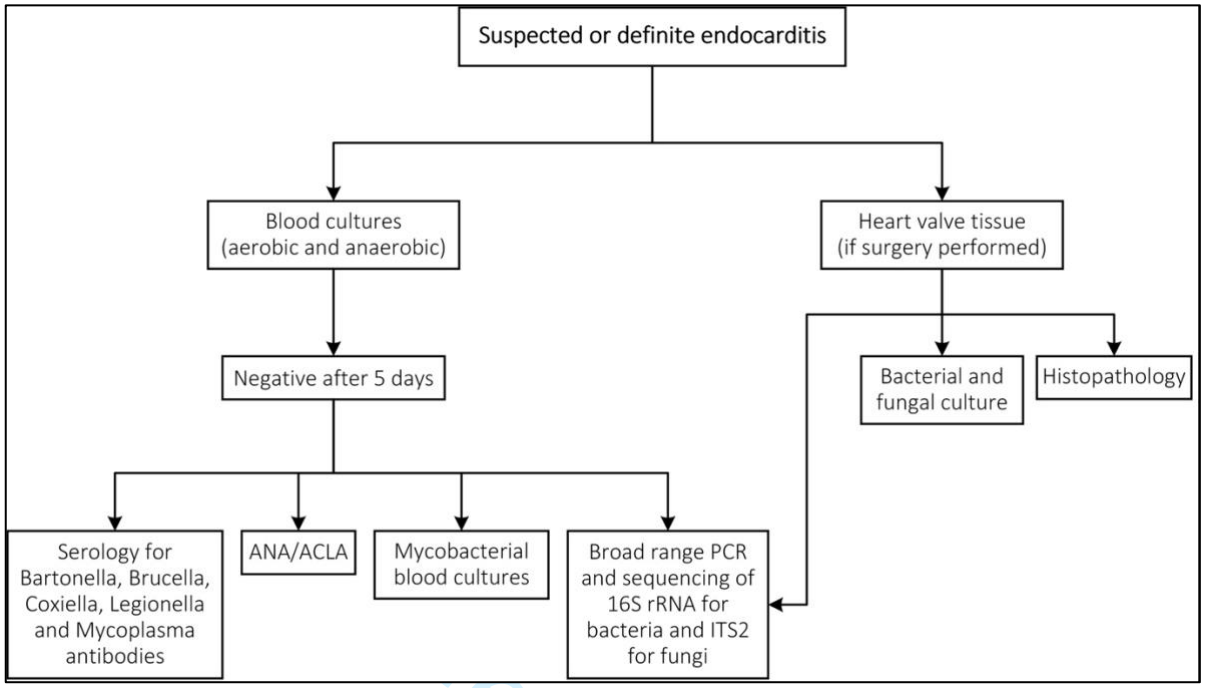
PLHIV – People living with Human Immunodeficiency Virus; c-ART – combination anti-retroviral therapy

Table 4: Results of set protocol for organism detection in patients with BCNIE in the prospective cohort

Test performed	n=28
Mycobacterium specific blood cultures positive	0
Anti-nuclear antibodies positive (ANA)	1
Serology indicating acute infection	15 (53.5%)
• *Bartonella species*	13
• *Mycoplasma species*	2
Blood PCR positive	1/22 (4.5%)
• Mycoplasma hominis	1
Heart valve PCR positive	9/20 (45%)
• *Bartonella quintana*	6

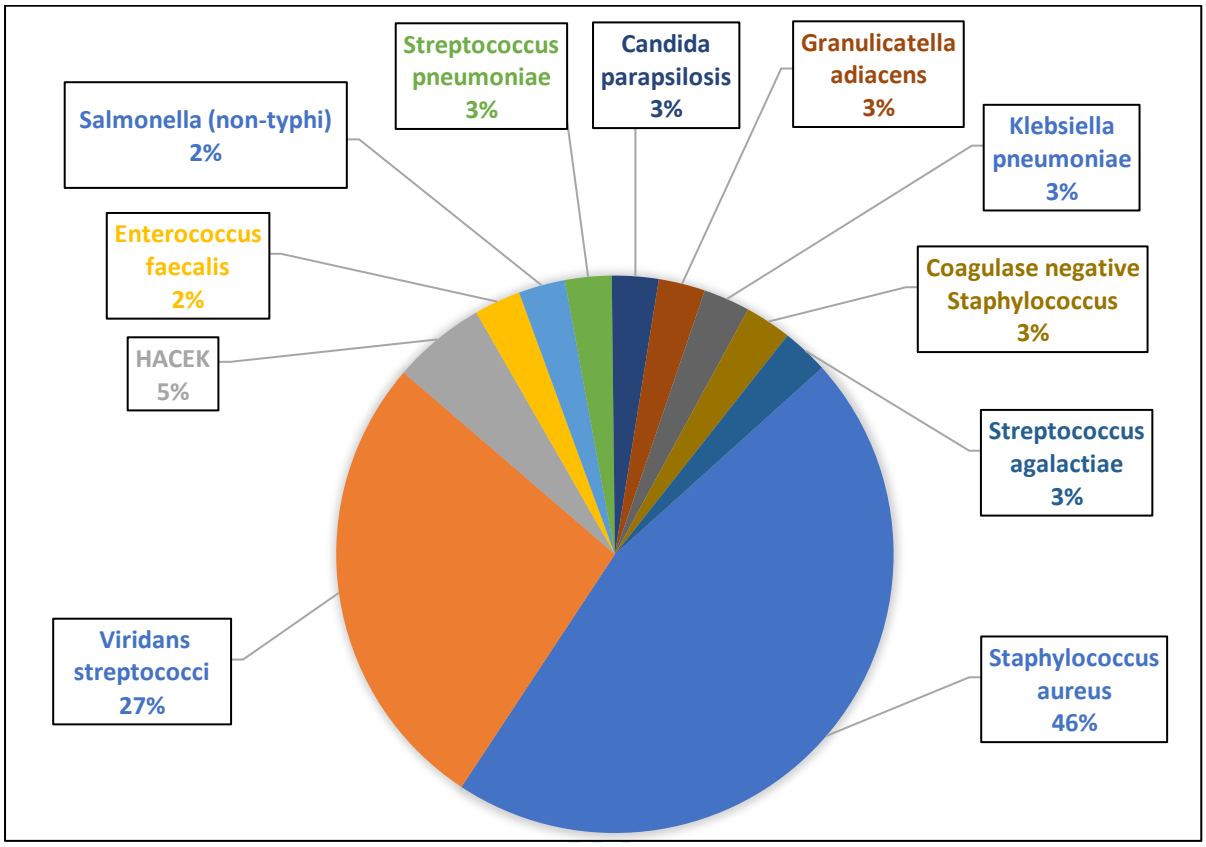
	• *Bartonella henselae*	1
	• *Mycoplasma hominis*	1
	• *Alternaria species*	1
Histopathological confirmed IE		20/20 (100%)
	• Cause identified (*Mycobacterium tuberculosis*)	1/20
Heart valve culture positive		0 / 20

Figure 1: Protocol for organism detection



ANA – antinuclear factor; ACLA – anti-cardiolipin antibodies; PCR – polymerase chain reaction

Figure 2: Causes of BCPIE (%)



*HACEK - Haemophilus, Aggregatibacter, Cardiobacterium, Eikenella, Kingella

Figure 3: Causes of BCNIE (%) detected by non-culture dependent techniques

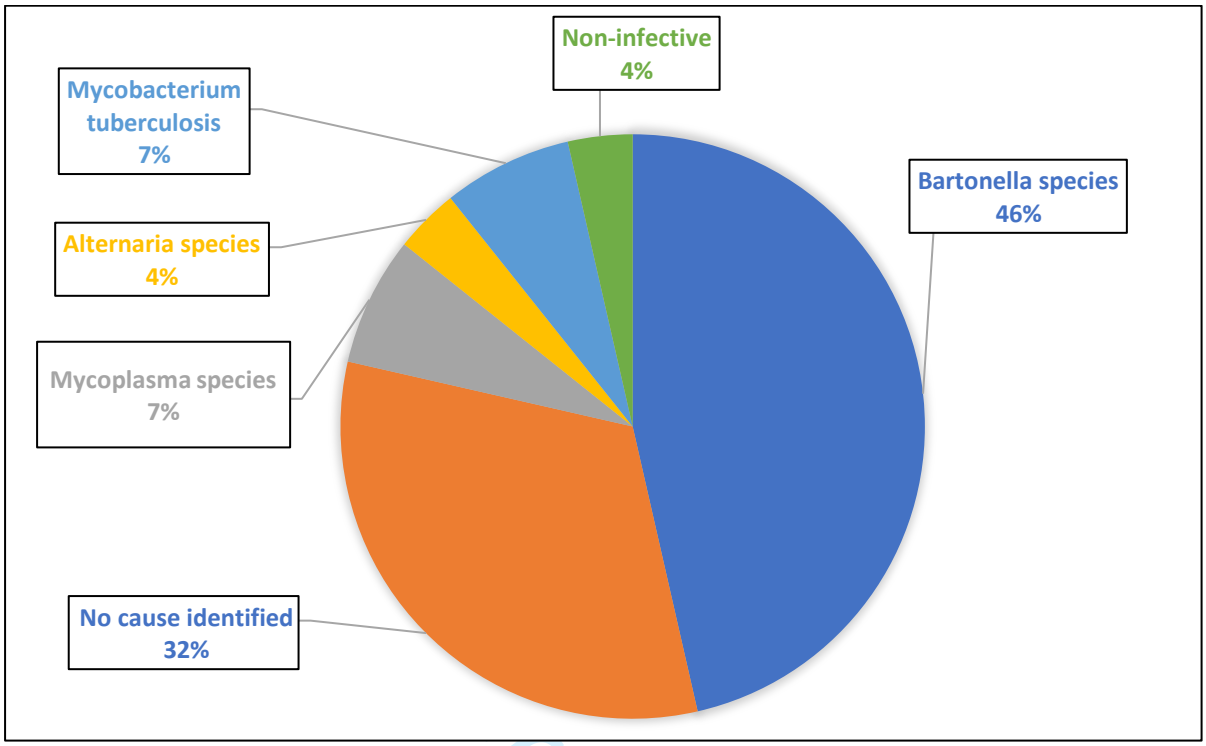
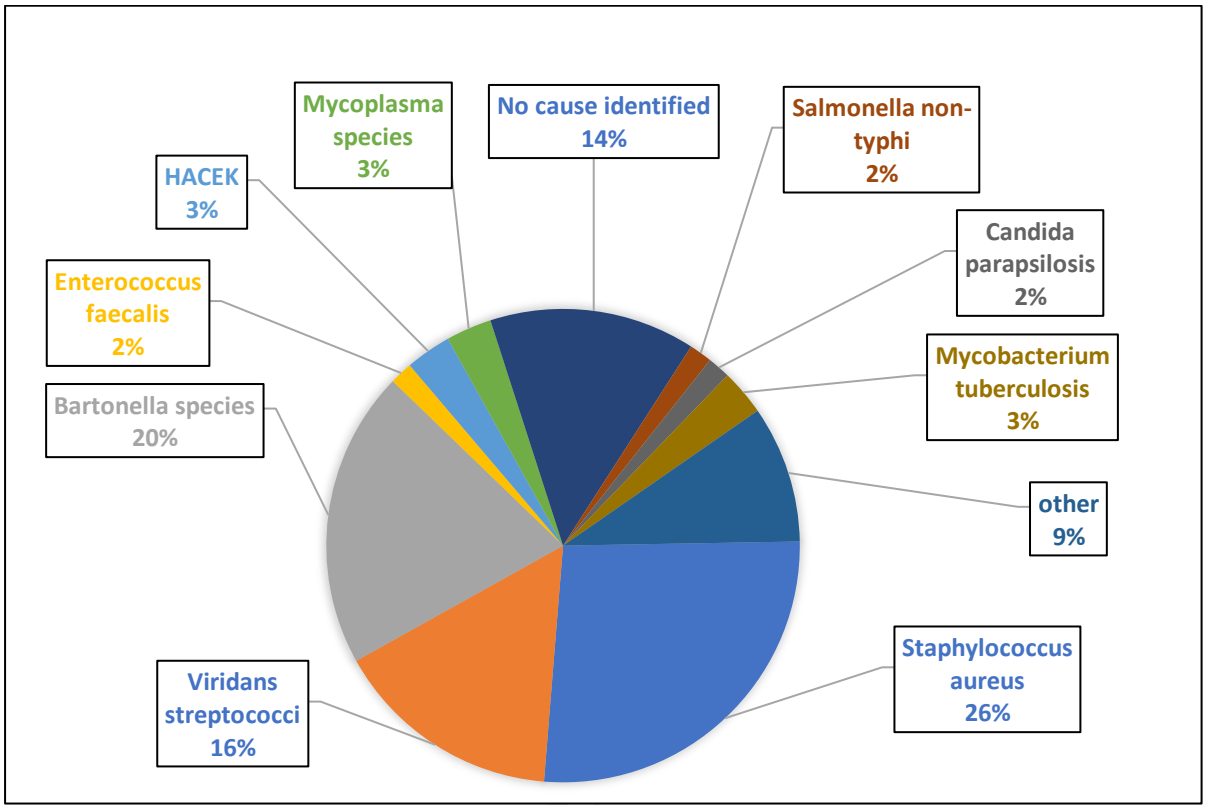


Figure 4: Causes of IE after set protocol for organism detection



*HACEK - Haemophilus, Aggregatibacter, Cardiobacterium, Eikenella, Kingella

Addendum A: Stepwise approach to identify causative organism or non-infective cause

A stepwise approach was employed to identify the causative organisms of IE and to minimise the incidence of BCNIE. At least three sets of blood cultures per patient were collected using an aseptic technique. Each set was drawn from a different peripheral site and included a BacT/ALERT® FA Plus (aerobic) (bioMérieux, Marcy l'Étoile, France) and BacT/ALERT® FN Plus (anaerobic) bottle. Additional blood cultures were obtained if clinical features of infection persisted. The blood cultures were submitted to the Microbiology laboratory of the National Health Laboratory Service (NHLS) at Tygerberg Hospital, and incubated in the BacT/ALERT® 3D automated microbial detection system for five days. Once the instrument detected growth in the bottles and signalled them as positive, they were removed, a Gram stain performed and the clinician immediately phoned and informed of the result. Depending on the organism observed on Gram stain, the blood culture broth was sub-cultured onto appropriate solid culture media such as blood agar, chocolate agar and MacConkey agar (prepared in-house), followed by incubation at the required temperature and atmosphere to optimise growth. The agar plates were examined after 18-24 hours of incubation and isolates identified using manual and/or automated biochemical assays; if these methods failed, isolates were referred for mass spectrometry (VITEK® MS, bioMérieux, Marcy l'Étoile, France). Antimicrobial susceptibility testing (AST) was performed by disk diffusion, automated broth dilution (VITEK® 2, bioMérieux, Marcy l'Étoile, France) and/or Etest® (bioMérieux, Marcy l'Étoile, France). AST results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) criteria. If no organism was isolated after five days using standard culture techniques, patients were defined as having BCNIE. Further testing performed on these patients are summarised in Figure 1 and included:

- Serology: Indirect immunofluorescence assays (IFA) for detection of antibodies to *Bartonella henselae* and *B. quintana* (Bartonella IFA IgM and IgG kits, FOCUS Diagnostics, Cypress, CA, USA), and *C. burnetii* (Q Fever IgM and IgG kits, FOCUS Diagnostics, Cypress, CA, USA). Enzyme-linked immunosorbent assays (ELISA) were performed to detect IgM and IgG antibodies to *Brucella* species (MASTAZYME BRUCELLA kit, MAST DIAGNOSTICA, Reinfeld, Germany), *Legionella pneumophila* (EUROIMMUN, Lübeck, Germany) and *Mycoplasma pneumoniae* (EUROIMMUN, Lübeck, Germany).
- Auto-antibody testing: Antinuclear antibodies (ANA) were detected with the Kallestad® HEp-2 Cell Line Substrate and Kallestad Mouse Stomach/Kidney Test kits (Bio-Rad Laboratories Inc, Redmond, WA, USA), and anti-cardiolipin antibodies (ACLA) with the QUANTA Lite PR3 SC and MPO SC ELISA kits (Inova Diagnostics, San Diego, CA, USA).

- Molecular testing on negative blood cultures: DNA was extracted from 200 µl of blood culture broth using the tissue protocol of the Qiagen QIAamp DNA Mini Kit, following benzyl alcohol extraction, as previously described. (1) Bacterial 16S rRNA PCR amplification was performed using primers BAK11w and BAK2 (2) and KAPA2G Robust HotStart ReadyMix (Kapa Biosystems, South Africa) according to manufacturer's instructions, with an annealing temperature of 55°C for 30 cycles. Fungal ITS2 amplification was performed using primers ITS1 and ITS4 (3) and KAPA Taq ReadyMix (Kapa Biosystems) in a touchdown PCR with annealing temperatures of 56°C for 10 cycles and 54°C for 30 cycles. Amplicons were sequenced on the ABI 3500XL genetic analyser at Inqaba Biotec (South Africa). Bacterial and fungal identification was based on >99% sequence alignment to published sequences available in the National Center for Biotechnology Information's Genbank database.
- Mycobacterial blood cultures: BACTEC™ Myco/F Lytic Culture vials (Becton Dickinson, Sparks, MD, USA) were collected for the isolation of *Mycobacterium tuberculosis* (MTB) and non-tuberculous Mycobacteria. The bottles were incubated in an automated continuous monitoring BACTEC 9120 instrument for 42 days. Work-up of positive cultures is not included since all cultures were negative.

If surgery was performed, heart valve tissue was submitted for:

- Bacterial and fungal culture
- Broad range PCR and sequencing of 16S rRNA for bacteria and ITS2 for fungi
- Histopathologic examination for detection of bacteria and fungi, as well as histopathological features of IE.

References:

1. Christensen *et al*. (2003). Rapid Identification of Bacteria from Positive Blood Cultures by Terminal Restriction Fragment Length Polymorphism Profile Analysis of the 16S rRNA Gene. *J. Clin. Microbiol.* 41:3790-3800
2. Bosshard *et al*., 2006. 16S rRNA Gene Sequencing verses the API 20 NE system and the VITEK 2 ID-GNB Card for Identification of Non-fermenting Gram-Negative Bacteria in the Clinical Laboratory. *J. Clin. Microbiol*. 44: 1359-1366
3. White *et al*., (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols: a guide to methods and applications. (Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds). Academic Press, New York, USA: 315–322

STROBE Statement— Causes of infective endocarditis in the Western Cape, South Africa

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	2
Objectives	3	State specific objectives, including any prespecified hypotheses	2
Methods			
Study design	4	Present key elements of study design early in the paper	3
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	3
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	3
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	3
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*4*
Bias	9	Describe any efforts to address potential sources of bias	4
Study size	10	Explain how the study size was arrived at	n/a
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	4
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	4
		(*b*) Describe any methods used to examine subgroups and interactions	4
		(*c*) Explain how missing data were addressed	n/a
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	n/a
		(*e*) Describe any sensitivity analyses	

Continued on next page

Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	5
		(b) Give reasons for non-participation at each stage	n/a
		(c) Consider use of a flow diagram	n/a
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	5
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	*5*
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	5
		(*b*) Report category boundaries when continuous variables were categorized	n/a
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n/a
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	5
Discussion			
Key results	18	Summarise key results with reference to study objectives	6-8
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	8
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	8
Generalisability	21	Discuss the generalisability (external validity) of the study results	8-9
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	9

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.